

Test Facility : IIVS
 Chemical Code : N/A
 2nd Chem. Code*: NRU

Study Number.: R&D - NR Stain Time Course in 3T3
 96-Well Plate ID : 1
 Experiment ID : RD96023T

96-WELL PLATE MAP												
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank							
B	Blank											
C	Blank											
D	Blank	3 hr	3 hr	2 hr	2 hr	1 hr	1 hr	30 min	30 min	15 min	15 min	
E	Blank											
F	Blank											
G	Blank											
H	Blank	Blank	Blank	Blank	Blank							

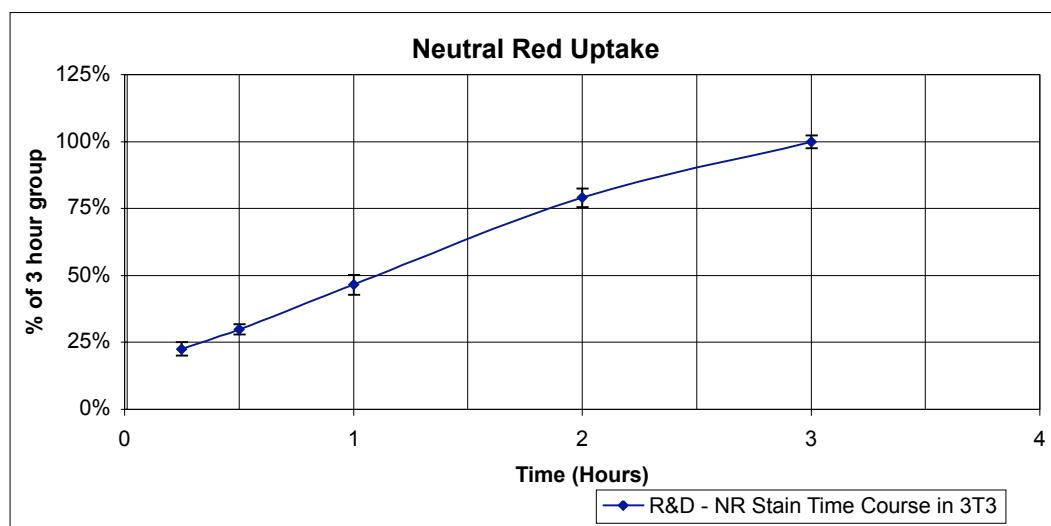
RAW ABSORBANCE DATA (OD ₅₅₀)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.048	0.046	0.045	0.047	0.047	0.046	0.046	0.044	0.044	0.043	0.044	0.038
B	0.048	0.753	0.794	0.595	0.607	0.415	0.396	0.267	0.282	0.219	0.213	0.039
C	0.047	0.866	0.766	0.668	0.668	0.406	0.391	0.257	0.256	0.227	0.220	0.038
D	0.046	0.844	0.794	0.607	0.622	0.393	0.387	0.228	0.262	0.213	0.217	0.038
E	0.046	0.717	0.805	0.627	0.610	0.384	0.375	0.239	0.266	0.210	0.206	0.038
F	0.044	0.776	0.769	0.618	0.665	0.378	0.398	0.277	0.301	0.186	0.202	0.038
G	0.043	0.717	0.807	0.639	0.616	0.385	0.349	0.265	0.269	0.211	0.195	0.036
H	0.044	0.044	0.045	0.044	0.045	0.045	0.043	0.043	0.045	0.045	0.041	0.036

CORRECTED ABSORBANCE (Sample OD ₅₅₀ - Mean Blank OD ₅₅₀)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.005	0.003	0.002	0.004	0.004	0.003	0.003	0.001	0.001	0.000	0.001	-0.005
B	0.005	0.710	0.751	0.552	0.564	0.372	0.353	0.224	0.239	0.176	0.170	-0.004
C	0.004	0.823	0.723	0.625	0.625	0.363	0.348	0.214	0.213	0.184	0.177	-0.005
D	0.003	0.801	0.751	0.564	0.579	0.350	0.344	0.185	0.219	0.170	0.174	-0.005
E	0.003	0.674	0.762	0.584	0.567	0.341	0.332	0.196	0.223	0.167	0.163	-0.005
F	0.001	0.733	0.726	0.575	0.622	0.335	0.355	0.234	0.258	0.143	0.159	-0.005
G	0.000	0.674	0.764	0.596	0.573	0.342	0.306	0.222	0.226	0.168	0.152	-0.007
H	0.001	0.000	0.002	0.001	0.002	0.002	0.000	0.000	0.002	0.002	-0.002	-0.007

Mean Blank = 0.043

RELATIVE VIABILITY (% OF VEHICLE CONTROL)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	95.8%	101.4%	74.5%	76.1%	50.2%	47.6%	30.2%	32.2%	23.7%	22.9%		
B	111.1%	97.6%	84.3%	84.3%	49.0%	46.9%	28.9%	28.7%	24.8%	23.9%		
C	108.1%	101.4%	76.1%	78.1%	47.2%	46.4%	24.9%	29.5%	22.9%	23.5%		
D	91.0%	102.8%	78.8%	76.5%	46.0%	44.8%	26.4%	30.1%	22.5%	22.0%		
E	98.9%	98.0%	77.6%	83.9%	45.2%	47.9%	31.6%	34.8%	19.3%	21.4%		
F	91.0%	103.1%	80.4%	77.3%	46.1%	41.3%	29.9%	30.5%	22.6%	20.5%		

Conc. (ug/mL) :	3 hr	3 hr	2 hr	2 hr	1 hr	1 hr	30 min	30 min	15 min	15 min	
Mean Corr. OD :	0.736	0.746	0.582	0.588	0.350	0.339	0.212	0.229	0.168	0.166	
SD :	0.064	0.018	0.026	0.028	0.014	0.018	0.019	0.016	0.014	0.010	
Mean 3 hour :	0.741										
Mean Blank :	0.043										
% of 3 hour:	99.3%	100.7%	78.6%	79.4%	47.3%	45.8%	28.6%	31.0%	22.6%	22.3%	
SD :	8.6%	2.4%	3.5%	3.7%	1.9%	2.5%	2.5%	2.2%	1.9%	1.3%	
% CV :	8.63%	2.37%	4.42%	4.72%	4.08%	5.42%	8.73%	7.14%	8.22%	5.76%	
hours			3	2	1	0.50	0.25				
% of 3 hour:			100.0%	79.0%	46.5%	29.8%	22.5%				



Neutral Red Stain Prepared in DMEM5%NCS - TEST OF NR PREP 1 DAY PRIOR TO USE
Tested in 90-100% Confluent 3T3 Cultures

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank											Blank
C	Blank				50 ug/ml							Blank
D	Blank			Prepared and filtered in evening before use		50 ug/ml						Blank
E	Blank					Filtered before use						Blank
F	Blank											Blank
G	Blank											Blank
H	Blank	Blank	Blank		Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD₅₅₀)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.045	0.045	0.045	0.044	0.056	0.056	0.056	0.057	0.053	0.051	0.051	0.052
B	0.043	0.383	0.459	0.417	0.541	0.631	0.639	0.635	0.637	0.686	0.656	0.052
C	0.045	0.389	0.397	0.379	0.557	0.536	0.621	0.559	0.590	0.618	0.612	0.051
D	0.043	0.383	0.429	0.350	0.539	0.575	0.545	0.629	0.613	0.658	0.652	0.053
E	0.042	0.361	0.345	0.334	0.579	0.585	0.577	0.573	0.626	0.635	0.599	0.051
F	0.044	0.368	0.412	0.374	0.582	0.588	0.578	0.572	0.687	0.647	0.641	0.050
G	0.042	0.415	0.451	0.422	0.600	0.620	0.616	0.632	0.572	0.744	0.637	0.050
H	0.044	0.042	0.043	0.043	0.057	0.059	0.055	0.057	0.050	0.057	0.050	0.054

CORRECTED ABSORBANCE (Sample OD₅₅₀ - Mean Blank OD₅₅₀)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.002	0.002	0.002	0.001	0.013	0.013	0.013	0.014	0.010	0.008	0.008	0.009
B	0.000	0.340	0.416	0.374	0.498	0.588	0.596	0.592	0.594	0.643	0.613	0.009
C	0.002	0.346	0.354	0.336	0.514	0.493	0.578	0.516	0.547	0.575	0.569	0.008
D	0.000	0.340	0.386	0.307	0.496	0.532	0.502	0.586	0.570	0.615	0.609	0.010
E	-0.001	0.318	0.302	0.291	0.536	0.542	0.534	0.530	0.583	0.592	0.556	0.008
F	0.001	0.325	0.369	0.331	0.539	0.545	0.535	0.529	0.644	0.604	0.598	0.007
G	-0.001	0.372	0.408	0.379	0.557	0.577	0.573	0.589	0.529	0.701	0.594	0.007
H	0.001	0.000	0.000	0.000	0.014	0.016	0.012	0.014	0.007	0.014	0.007	0.011

Mean Blank = 0.052 (Only the 14 wells from the 33 ug/ml group)

Conc. (µg/mL) :	Neutral Red Stain Concentration											
	50.0				50.0							
Mean Corr. OD :	0.340 0.372 0.336 0.523 0.546 0.553 0.557 0.578 0.621 0.590											
SD :	0.019 0.042 0.035 0.025 0.034 0.035 0.035 0.040 0.045 0.023											
Group mean corr OD:	0.349 0.545 0.596											

Note: Significant crystal formation was observed in the DMEM5%NCS/NR prepared 1 day prior, and the color was essentially medium-colored. Much NR stain stripped out of solution. No ppt or crystallization observed in the wells during the NR loading of cells.

Neutral Red Stain Prepared in DMEM5%NCS/Filtered immediately before use
Tested in 90-100% Confluent 3T3 Cultures

96-WELL PLATE MAP												
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	
B	Blank	50 ug/ml	50 ug/ml	28 ug/ml	28 ug/ml	16 ug/ml	16 ug/ml	9 ug/ml	9 ug/ml	5 ug/ml	5 ug/ml	
C	Blank											
D	Blank											
E	Blank											
F	Blank											
G	Blank											
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Empty

RAW ABSORBANCE DATA (OD ₅₅₀)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.076	0.051	0.05	0.045	0.044	0.041	0.041	0.041	0.039	0.038	0.037	0.037
B	0.058	0.553	0.535	0.58	0.587	0.421	0.353	0.225	0.221	0.149	0.145	0.037
C	0.053	0.561	0.503	0.517	0.549	0.338	0.345	0.213	0.203	0.144	0.155	0.035
D	0.048	0.493	0.527	0.489	0.495	0.351	0.331	0.196	0.196	0.143	0.161	0.038
E	0.047	0.491	0.497	0.528	0.571	0.312	0.321	0.188	0.195	0.132	0.172	0.038
F	0.073	0.606	0.697	0.53	0.6	0.36	0.373	0.239	0.218	0.143	0.163	0.036
G	0.072	0.63	0.497	0.563	0.592	0.399	0.39	0.235	0.21	0.145	0.157	0.037
H	0.056	0.089	0.055	0.043	0.045	0.041	0.04	0.039	0.039	0.042	0.04	0.036

CORRECTED ABSORBANCE (Sample OD ₅₅₀ - Mean Blank OD ₅₅₀)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.033	0.008	0.007	0.002	0.001	-0.002	-0.002	-0.002	-0.004	-0.005	-0.006	-0.006
B	0.015	0.510	0.492	0.537	0.544	0.378	0.310	0.182	0.178	0.106	0.102	-0.006
C	0.010	0.518	0.460	0.474	0.506	0.295	0.302	0.170	0.160	0.101	0.112	-0.008
D	0.005	0.450	0.484	0.446	0.452	0.308	0.288	0.153	0.153	0.100	0.118	-0.005
E	0.004	0.448	0.454	0.485	0.528	0.269	0.278	0.145	0.152	0.089	0.129	-0.005
F	0.030	0.563	0.654	0.487	0.557	0.317	0.330	0.196	0.175	0.100	0.120	-0.007
G	0.029	0.587	0.454	0.520	0.549	0.356	0.347	0.192	0.167	0.102	0.114	-0.006
H	0.013	0.000	0.012	0.000	0.002	-0.002	-0.003	-0.004	-0.004	-0.001	-0.003	-0.007

Neutral Red Stain Concentration												
Conc. (ug/mL) :	50.0	50.0	28.0	28.0	15.8	15.8	8.9	8.9	5.0	5.0		
Mean Corr. OD :	0.512	0.499	0.491	0.522	0.320	0.309	0.173	0.164	0.099	0.116		
SD :	0.057	0.077	0.033	0.039	0.040	0.026	0.021	0.011	0.006	0.009		
Group mean corr OD:	0.506		0.507		0.315		0.168		0.107			
	graph	x	50.0	28.0	15.8	8.9	5.0					
	y		0.506	0.507	0.315	0.168	0.107					

